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APPLICATION NUMBER: *60/546,227*
FILING DATE: *February 20, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US05/05626*

Certified by

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV222768245US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Robert	Danziger	Chicago, Illinois

Additional inventors are being named on the _____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

Inhibiting PDE4B and PDE4D Activity Reduces Blood Pressue in Salt-Sensitive Hypertension

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 10 ☐ CD(s), Number _____

☐ Drawing(s) *Number of Sheets* _____ ☐ Other (specify) _____

☐ Application Data Sheet. See 37 CFR 1.76

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☑ Applicant claims small entity status. See 37 CFR 1.27.

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FILING FEE Amount ($): $80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.

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Respectfully submitted,

SIGNATURE _____ Date February 20, 2004

TYPED or PRINTED NAME Gordon Comstock REGISTRATION NO. _____ *(if appropriate)*

TELEPHONE 312-996-7779 Docket Number: CW049

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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Gordon L. Comstock
Director, Medicine Intellectual Property

Provisional Patent Application of

Robert S. Danziger

for

INHIBITING PDE4B AND PDE4D ACTIVITY REDUCES BLOOD PRESSURE IN SALT-SENSITIVE HYPERTENSION

BACKGROUND

There is evidence that increased levels of cyclic adenosine monophosphate (cAMP) both reduces renal vascular resistance and promotes secretion of electrolytes. However, the role that the metabolism of cAMP plays in physiological salt-adaptation and hypertension is not known. We show that PDE4B and PDE4D inhibition reduces blood pressure in a model of salt-sensitive hypertension.

Dibutyryl cAMP increases both renal blood flow and urinary Na+ excretion. Relaxant properties of cAMP and beta-adrenergic signaling in arterial beds and in the renal resistance arteries in particular are well established [1,2] . Inhibitors of cAMP-dependent protein kinase (PkA) and the Rp diastereomer of adenosine 3',5'-cyclic monophosphorothioate block fluid secretion of both fluids and electrolytes from the inner medullary

collecting ducts (IMCD)[3]. cAMP agonists inhibit renal Na/K ATPase activity via a PkA-phospholipase A2 [4,5]. However, the role that renal cAMP plays in physiological salt-adaptation and hypertension is not known. The following work shows that renal cAMP mediates salt-adaptation and plays a mechanistic role in salt-sensitive hypertension.

The components of beta-adrenergic signaling pathway, which is believed to be the primary source of cellular cAMP, are known. Beta-adrenergic agonists stimulate, through G-protein mediated coupling, adenylate cyclase, which converts ADP to cAMP. cAMP in turn stimulates specific protein kinases, i.e., PkAs, that phosphorylate a variety of proteins. Specific PDEs metabolize cAMP to adenosine monophosphate. Thus, PDEs are in central position to regulate cAMP signaling. However, the role that PDEs play in normal physiological processes in general and specifically in salt-adaptation and hypertension are largely known. We answer this in the present work by showing that renal PDEs plays a mechanistic role in blood pressure control.

The cyclic nucleotide PDEs comprise a large number of different isozymes with variable specificities for cyclic guanosine monophosphate (cGMP) and cAMP. The PDE4 family is characterized by a specificity and high affinity for cAMP and consists of four individual isogenes, PDE4A, 4B, 4C, and 4D [10,11]. Three of these have been shown to express multiple proteins by virtue of alternate splicing and start sites [12-14]. PDE4 activity

has been reported in kidney, gonads, cardiac muscle, liver, tracheal smooth muscle, various regions of the brain, and in inflammatory cells. Specific PDE4B splice variant have been found to be preferentially expressed in the membrane (PDE4B2) and the cytosol (PDE4B1) of the brain [15]. These two isoforms differ in their N-terminal domains, suggesting that differences in this domain can determine subcellular localization of the protein [16]. However, the physiological significance of the different isoforms is unknown. In our work, a function of renal PDE4B and/or PDE4D, is demonstrated.

Our work shows that PDE4, specifically PDE4B and PDE4D are therapeutic targets for salt-sensitive hypertension and that inhibiting PDE4 activity reduces blood pressure in salt-sensitive hypertension. This represents an important advance from the work of Tanahashi et al [26], which shows that in the dog kidney, PDE4 participates in the degradation of cAMP and that infusion directly into the renal artery of rolipram, an inhibitor of PDE4, enhances glomerular filtration and urinary Na+ excretion, by 1) examining rolipram in salt-adaptation and salt-sensitive hypertension; 2) using an intraperitoneal (leading to systemic) delivery method for rolipram; 3) demonstrating that rolipram is a potent anti-hypertensive agent; and 4) discovering that renal PDE4B and PDE4D are the targets for rolipram in hypertension.

Several therapeutic targets and agents have been identified for hypertension, e.g., angiotensin converting enzyme (ACE) and

ACE inhibitors; angiotensin and angiotensin receptor blockers; aldosterone and aldosterone antagonists; and central nervous system and methyl-DOPA. The present work advances the treatment of hypertension by discovering a new therapeutic target for the treatment of hypertension. Agents which act on the target, PDE4B, may be useful alone and/or provide additional efficacy to existing combination therapy.

The present discovery that PDE4 is a therapeutic target for hypertension leads to new uses for drugs and compounds that inhibit it. Thus, in addition to being of potential utility for disease such as COPD and asthma, they may be useful for arterial hypertension and salt-sensitive hypertension.

DESCRIPTION

Fig. 1 is the continuous telemetric blood pressure monitoring for 6 days in a single Dahl salt-sensitive/Jr rat, a genetic model of renal salt-sensitivity, on 8% NaCl diet with single intraperitoneal injection of rolipram (10 mg/kg) at day 2 (arrow).

Fig. 2 shows the average blood pressures during continuous telemetric monitoring of Dahl SS/Jr rats on 8% NaCl diet before and after a single intraperitoneal injection of rolipram (10mg/kg).

Figures 1 and 2 show that a single injection rolipram causes a very significant and prolonged (> 2 days) drop in blood

pressure. Since rolipram inhibits PDE4 and the Dahl SS/Jr possesses renal salt-sensitive hypertension, this links PDE4 activity to salt-sensitive hypertension.

Fig. 3 is the renal PDE4B abundance in Dahl SR and SS rats on 8% and 0.3% NaCl diets for 10 days. Representative Western analysis using a PDE4B anti rabbit polyclonal antibody to PDE4 which detects PDE4B and splice variants showing greatest abundance of PDE4B1 splice variant.

Fig. 4 is a bar graph showing abundance of PDE4B1 determined in Dahl SS/Jr and salt-resistant (SR) rat kidneys by Western analysis using a polyclonal anti-rabbit antibody which reacts with various PDE4B splice variants ($n = 3$; $P < 0.05$).

Figures 3 and 4 show that PDE4B, a primary target of rolipram in general, is in the kidney of the Dahl SS/Jr rat, demonstrating that this isoform is a target of rolipran in the kidney of the Dahl SS/Jr rat. Figure 4 shows specifically that PDE4B1 abundance is influenced by dietary salt content, suggesting that it plays a mechanistic role in salt-adaptation and is a primary target for rolipram in reducing blood pressure.

Fig. 5 is a representative Western blot using PDE4D anti-rabbit antibody which detects PDED splice variants showing the presence of PDE4D5 in the kidney. Abundance is less in Dahl SR

kidney from rat on 8% versus 0.3% NaCl diets. This demonstrates that PDE4D5 is present in the rat kidney and appears to play a mechanistic role in salt adaptation. Thus PDE4D5 is also a renal target of rolipram.

Figure 1

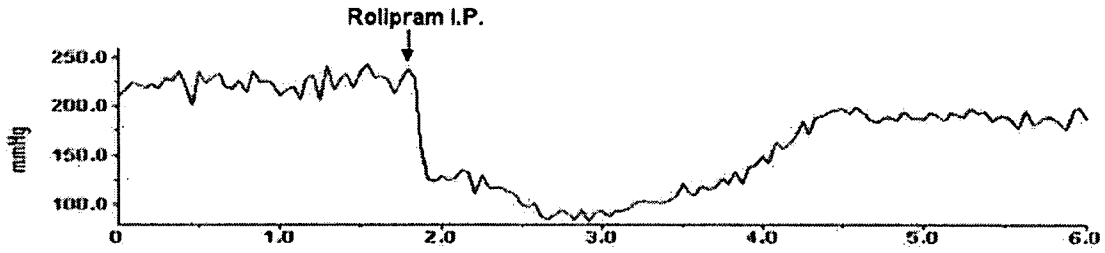

Figure 2

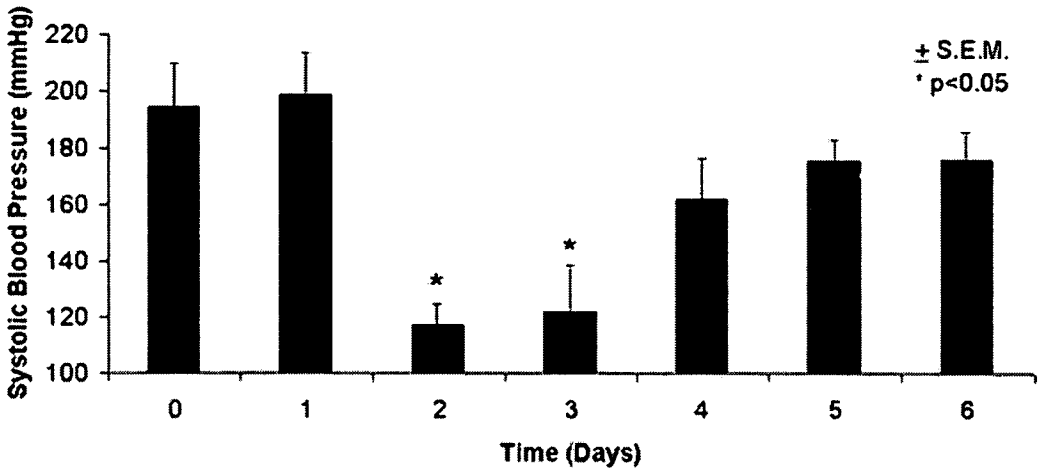
Systolic Blood Pressure (mmHg)
220
200
180
160
140
120
100
0
1
2
3
4
5
6
Time (Days)
± S.E.M.
* p<0.05
*
*

Figure 3

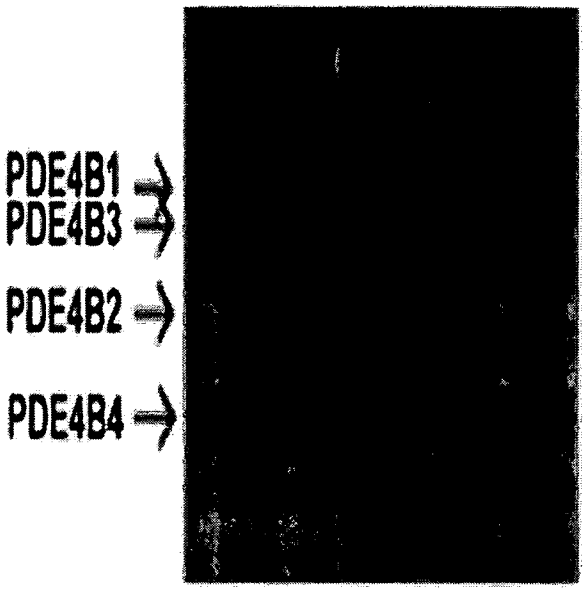

Figure 4

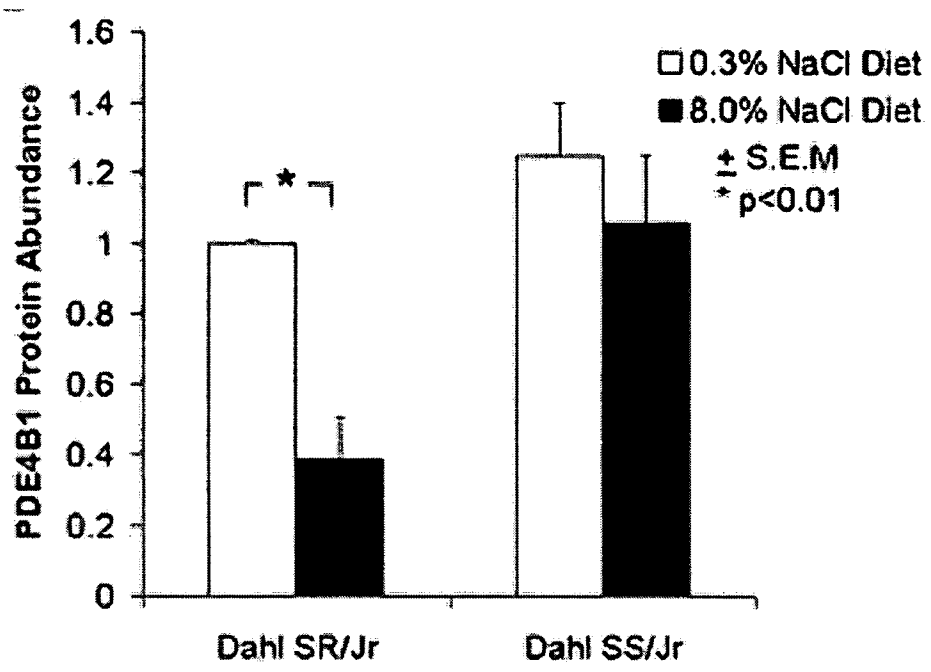

Figure 5

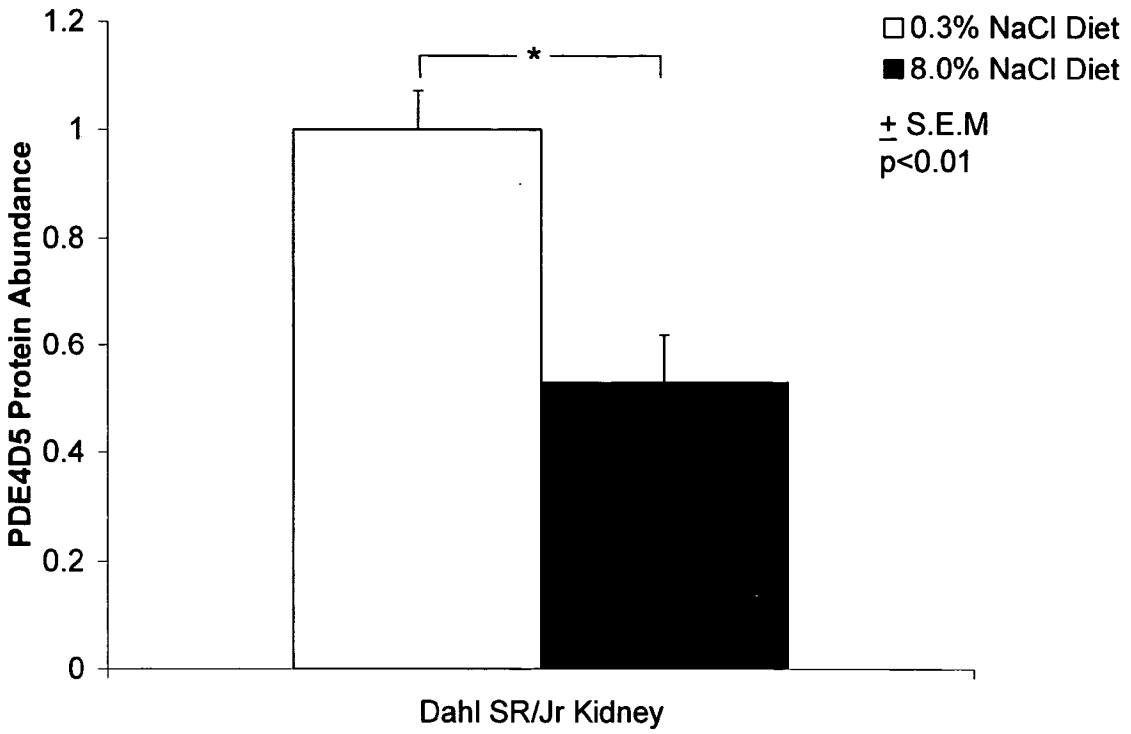
1.2
1
0.8
0.6
0.4
0.2
0
PDE4D5 Protein Abundance
*
0.3% NaCl Diet
8.0% NaCl Diet
± S.E.M
p<0.01
Dahl SR/Jr Kidney

Figure 5